

MOUSE *Fkh^f* cDNA SEQUENCE

1 GCTGATCCCC CTCTAGCA GT CCACTTCACC AAGGTGAGCG AGTGTCCCTG
 51 CTCTCCCCCA CCAGACACAG CTCTGCTGGC GAAAGTGGCA GAGAGGTATT
 101 GAGGGTGGGT GTCAGGAGCC CACCAAGTACA GCTGGAAACA CCCAGCCACT
 151 CCAGCTCCCC GCAACTTCTC CTGACTCTGC CTTCAAGACGA GACTTGGAAAG
 201 ACAGTCACAT CTCAGCAGCT CCTCTGCCGT TATCCAGCCT GCCTCTGACA
 251 AGAACCCAAT GCCCAACCCCT AGGCCAGCCA AGCCTATGGC TCCTTCCTTG
 301 GCCCTTGGCC CATCCCCAGG AGTCTTGCCA AGCTGGAAGA CTGCACCCAA
 351 GGGCTCAGAA CTTCTAGGGA CAAGGGGCTC TGGGGGACCC TTCCAAGGTC
 401 GGGACCTGCG AAGTGGGGCC CACACCTCTT CTTCCCTGAA CCCCCTGCCA
 451 CCATCCCAGC TGCACTGCC TACAGTGCCC CTAGTCATGG TGGCACCGTC
 501 TGGGGCCCGA CTAGGTCCCT CACCCCCACCT ACAGGCCCTT CTCCAGGACA
 551 GACCACACTT CATGCATCAG CTCTCCACTG TGGATGCCCA TGCCCAGACCC
 601 CCTGTGCTCC AAGTGCCTCC ACTGGACAAC CCAGCCATGA TCAGCCTCCC
 651 ACCACCTTCT GCTGCCACTG GGGTCTTCTC CCTCAAGGCC CGGCCTGGCC
 701 TGCCACCTGG GATCAATGTG GCCAGTCTGG AATGGGTGTC CAGGGAGCCA
 751 GCTCTACTCT GCACCTTCCC ACGCTCGGGT ACACCCAGGA AAGACAGCAA
 801 CCTTTTGGCT GCACCCCAAG GATCCTACCC ACTGCTGGCA AATGGAGTCT
 851 GCAAGTGGCC TGGTTGTGAG AAGGTCTTCG AGGAGGCCAGA AGAGTTTCTC
 901 AAGCACTGCC AAGCAGATCA TCTCTGGAT GAGAAAGGCC AGGCCAGTG
 951 CCTCCCTCCAG AGAGAAGTGG TGCAGTCTCT GGAGCAGCAG CTGGAGCTGG
 1001 AAAAGGAGAA GCTGGGAGCT ATGCAGGCC ACCTGGCTGG GAAGATGGCG
 1051 CTGGCCAAGG CTCCCATCTGT GGCCTCAATG GACAAGAGCT CTTGCTGCR
 1101 CGTAGGCCAC AGTACTCAGG GGAGTGTGCT CCCGGCTGG TCTGCTCCTC
 1151 GGGAGGCTCC AGACGGCGGC CTGTTGCAG TGCGGAGGCA CCTCTGGGG
 1201 AGCCATGGCA ATAGTTCCCTT CCCAGAGTTC TTCCACAACA TGGACTACTT
 1251 CAAAGTACCAAC AATATGCGAC CCCCTTTCAC CTATGCCACC CTTATCCGAT
 1301 GGGCCATCCT GGAAGCCCCG GAGAGGAGA GGACACTCAA TGAAATCTAC
 1351 CATTGGTTTA CTCGCATGTT CGCCTACTTC AGAAACCACC CGGCCACCTG
 1401 GAAGAATGCC ATCCGCCACA ACCTGAGCCT GCACAAGTGC TTTGTGCGAG
 1451 TGGAGAGCGA GAAGGGAGCA GTGTGGACCG TAGATGAATT TGAGTTTCGC
 1501 AAGAAGAGGA GCCAACGCC CRACAAAGTGC TCCAATCCCT GCCCTTGACC
 1551 TCAAAACCAA GAAAAGGTGG GCGGGGGAGG GGGCCAAAAC CATGAGACTG
 1601 AGGCTGTGGG GGCAAGGAGG CAAGTCCCTAC GTGTACCTAT GGAAACCGGG
 1651 CGATGATGTG CCTGCTATCA GGGCCTCTGC TCCCTATCTA GCTGCCCTCC
 1701 TAGATCATAT CATCTGCCCTT ACAGCTGAGA GGGGTGCCAA TCCCAGCCTA
 1751 GCCCCTAGTT CCAACCTAGC CCCAAGATGA ACTTTCCAGT CAAAGAGGCC
 1801 TCACAACCAAG CTATACATAT CTGCCTTGGC CACTGCCAAG CAGAAAGATG
 1851 ACAGACACCA TCCTAATATT TACTCAACCC AAACCCCTAAA ACATGAAGAG
 1901 CCTGCCTTGG TACATTGCGT AACTTTCAA GTTAGTCATG CAGTCACACCA
 1951 TGACTGCAGT CCTACTGACT CACACCCCAA AGCACTCACC CACAACATCT
 2001 GGAACCACGG GCACTATCAC ACATAGGTGT ATATAACAGAC CCTTACACAG
 2051 CAACAGCACT GGAACCTTCA CAATTACATC CCCCCAAACCC ACACAGGCAT
 2101 AACTGATCAT ACGCAGCCTC AAGCAATGCC CAAAATACAA GTCAGACACA
 2151 GCTTGTCAAG

Figure 1

MOUSE Fkh^f PROTEIN SEQUENCE

1 MPNPRPAKPM APSLALGPSP GVLPSWKTAP KGSELLGTRG SGGPFQGRDL
51 RSGAHTSSSL NPLPPSQLQL PTVPLVMVAP SGARLGPSPH LQALLQDRPH
101 FMHQLSTVDA HAQTPVLQVR PLDNPAMISL PPPSAATGVF SLKARPGLPP
151 GINVASLEWV SREPALLCTF PRSGTPRKDS NLLAAPQGSY PLLANGVCKW
201 PGCEKVFEEP EEFLKHCQAD HLLDEKGKAQ CLLQREVVQS LEQQLELEKE
251 KLGAMQAHLA GKMALAKAPS VASMDKSSCC IVATSTQGSV LPAWSAPREA
301 PDGGLFAVRR HLWGSHGNSS FPEFFHNMDY FKYHNMRPPF TYATLIRWAI
351 LEAPERQRTL NEIYHWFTRM FAYFRNHPAT WKNAIRHNLS LHKCFVRVES
401 EKGAVWTVD EFERKKRSQR PNKCSNPCP*

Figure 2

HUMAN *FKH*^f cDNA Sequence

1 GCACACACTC ATCGAAAAA ATTGGATT A TAGAAGAGA GAGGTCTGCG
51 GCTTCCACAC CGTACAGCGT GTTTTCTT CTCGGTATAA AAGCAAAGTT
101 GTTTTGATA CGTGACAGTT TCCCACAAGC CAGGCTGATC CTTTCTGTC
151 AGTCCACTTC ACCAAGCCTG CCCTTGGACA AGGACCCGAT GCCCAACCCC
201 AGGCCTGGCA AGCCCTCGC CCCTTCTTG GCCCTTGGCC CATCCCCAGG
251 AGCCTCGCCC AGCTGGAGGG CTGCACCCAA AGCCTCAGAC CTGCTGGGG
301 CCCGGGGCCC AGGGGAACC TTCCAGGGCC GAGATCTCG AGGCGGGGCC
351 CATGCCTCCT CTTCTTCTT GAACCCCATG CCACCATCGC AGCTGCAGCT
401 GCCCCACACTG CCCCTAGTCA TGGTGGCACCC CTCCGGGCA CGGCTGGGCC
451 CCTTGCCCCA CTTACAGGCA CTCCCTCAGG ACAGGCCACA TTTCATGCAC
501 CAGCTCTCAA CGGTGGATGC CCACGCCCGG ACCCCTGTGC TGCAGGTGCA
551 CCCCCCTGGAG AGCCAGCCA TGATCAGCCT CACACCACCC ACCACCGCCA
601 CTGGGGTCTT CTCCCTCAAG GCCCCGGCTG GCCTCCCACC TGGGATCAAC
651 GTGGCCAGCC TGGATGGGT TCCAGGGAG CGGGCACTGC TCTGCACCTT
701 CCCAAATCCC AGTGCACCCA GGAAGGACAG CACCCCTTCG GCTGTGCC
751 AGAGCTCTTA CCCACTGCTG GCAAATGGTG TCTGCAAGTG GCCCAGGATGT
801 GAGAAGGTCT TCGAAGAGCC AGAGGACTTC CTCAAGCACT GCCAGGGCGA
851 CCATCTCTG GATGAGAAGG CAGGGCACA ATGTCCTC CAGAGAGRA
901 TGGTACAGTC TCTGGAGCAG CAGCTGGTC TGGAGAAGGA AAAGCTGAGT
951 GCCATGCAGG CCCACCTGGC TGGAAAATG GCACTGACCA AGGCTTCATC
1001 TGTGGCATCA TCCGACAAGG GCTCTGCTG CATCGTAGCT GCTGGCAGCC
1051 AAGGCCCTGT CGTCCCAGCC TGGTCTGGCC CCCGGGAGGC CCCTGACAGC
1101 CTGTTTGCTG TCCGGAGGCA CCTGTGGGGT AGCCATGGAA ACAGCACATT
1151 CCCAGAGTTC CTCCACAACA TGGACTACTT CAAGTTCCAC AACATGCCAC
1201 CCCCTTTCAC CTACGCCACG CTACATCCGCT GGGCCATCCT GGAGGCTCCA
1251 GAGAACGCAGC GGACACTCAA TGAGATCTAC CACTGGTTCA CACGCATGTT
1301 TGCCTTCTTC AGAAACCATC CTGCCACCTG GAAGAACGCC ATCCGCCACA
1351 ACCTGAGTCT GCACAAGTGC TTTGTGCGGG TGGAGAGCGA GAAGGGGGCT
1401 GTGTGGACCG TGGATGAGCT GGAGTTCCGC AAGAAACCGA GCCAGAGGCC
1451 CAGCAGGTGT TCCAACCTA CACCTGGCCC CTGACCTCAA GATCAAGGAA
1501 AGGAGGATGG ACGAACAGGG GCAAACCTGG TGGGAGGCAG AGGTGGTGGG
1551 GGCAGGGATG ATAGGCCCTG GATGTGCCA CAGGGACCAA GAAAGTGAGGT
1601 TTCCACTGTC TTGCTGCCA GGGCCCCCTGT TCCCCCGCTG GCAGCCACCC
1651 CCTCCCCCAT CATATCCTTT GCCCCAAGGC TGCTCAGAGG GGCCCCGGTC
1701 CTGGCCCCAG CCCCCACCTC CGCCCCAGAC ACACCCCCCA GTCGAGCCCT
1751 GCAGCCAAAC AGAGCCCTCA CAACCAAGCCA CACAGAGCCT GCCTCAGCTG
1801 CTCGCACAGA TTACTTCAGG GCTGGAAAAG TCACACAGAC ACACAAAAATG
1851 TCACAATCCT GTCCCTCAC

Figure 3

HUMAN FKHst PROTEIN SEQUENCE

1 MPNPRPGKPS APSLALGPSP GASPSWRAAP KASDLLGARG PGGTFQGRDL
51 RGGAHASSSS LNPMPPSQLQ LPTLPLVMVA PSGARLGPLP HLQALLQDRP
101 HFMHQILSTVD AHARTPVLQV HPLESPAMIS LTPPTTATGV FSLKARPGLP
151 PGINVASLEW VSREPALLCT FPNPNSAPRKD STLSAVPQSS YPLL LANGVCK
201 WPGCEKVFEE PEDFLKHCQA DHLLDEKGRA QCLLQREMVQ SLEQQLVLEK
251 EKLSAMQAHL AGKMALTAKS SVASSDKGSC CIVAAAGSQGP VVPAWSGP
301 APDSLFAVRR HLWGSHGNST FPEFLHNMDY FKFHNMRFPPF TYATLIRWAI
351 LEAPEKQRTL NEIYHWFTRM FAFFRNHPAT WKNAIRHNLS LHKCFVRVES
401 EKGAVWTVD LEFRKKRSQR PSRCSNPTPG P*

Figure 4

Vector for generation of FKHsf Transgenic mice

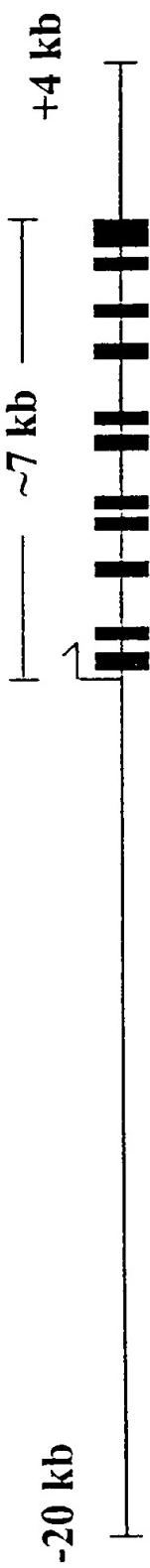


Figure 5

FKHsf Transgene corrects the defect in
scurfy animals

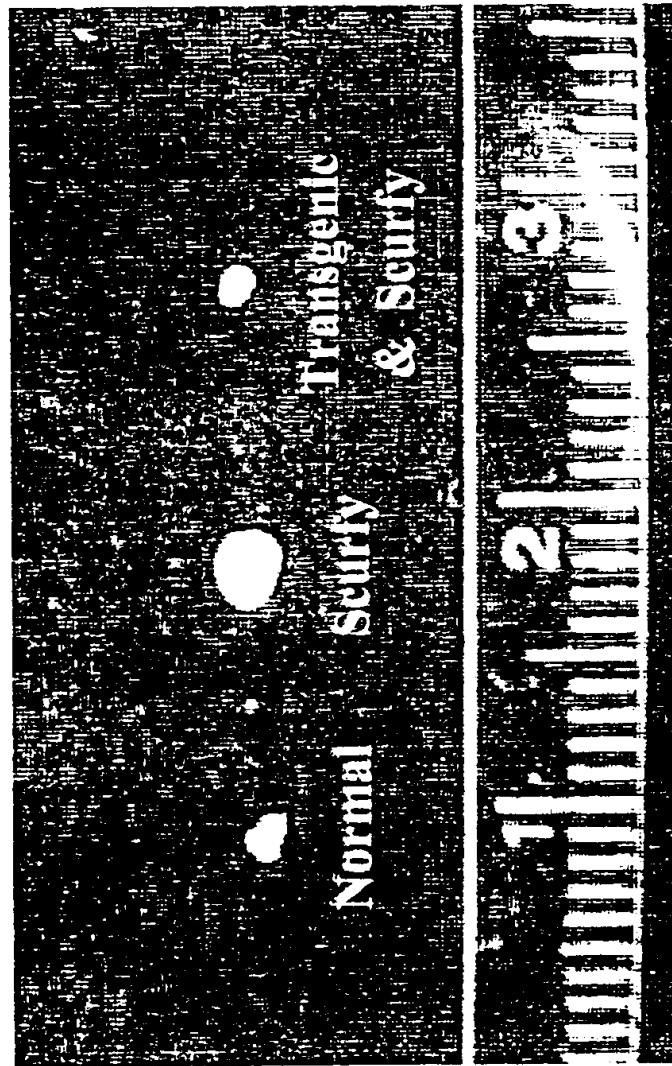


Figure 6

**FKHsf tg mice have reduced lymph node cells
compared to normal cells**

Cell number	Mouse genotype		
	Normal	Scurfy	Transgenic
Cells / LN	0.92	1.97	0.29
Cells / Thymus	0.76	0.54	0.76

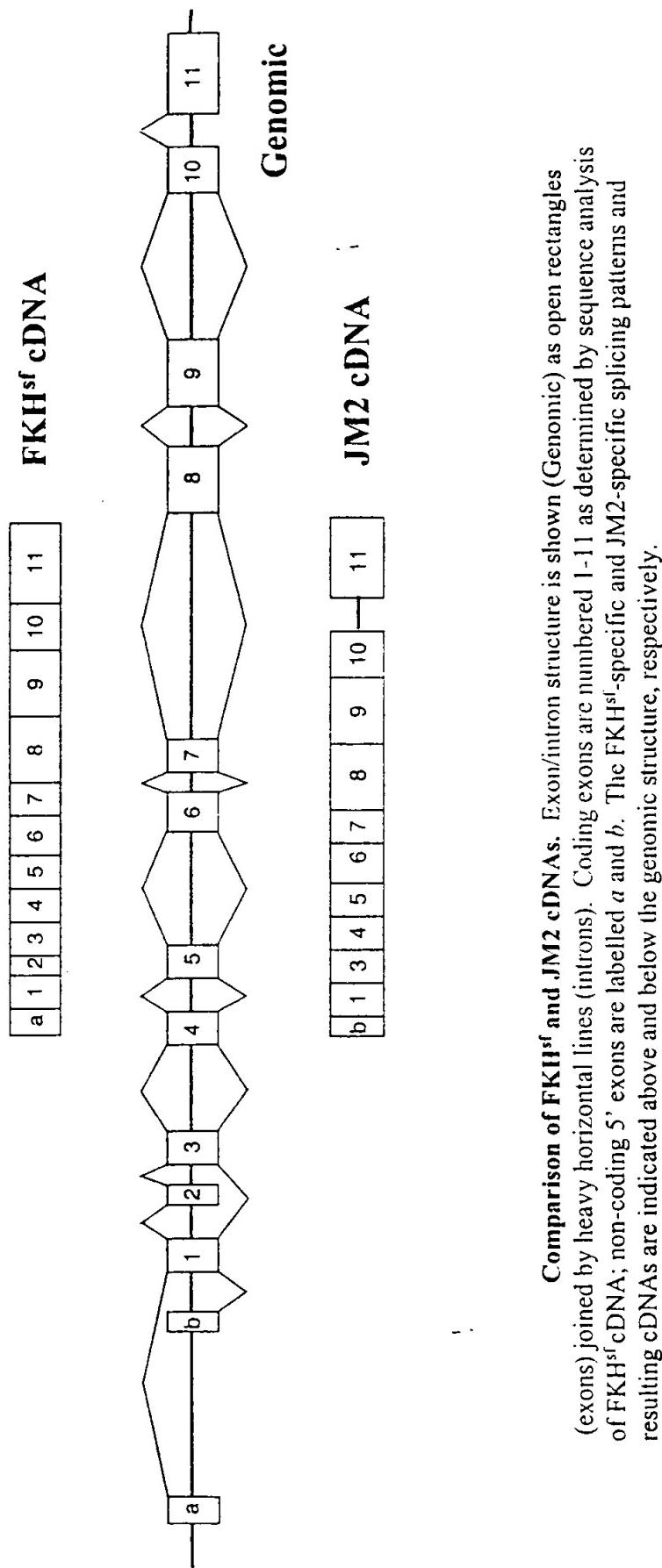
Figure 7

FKHsf transgenic mice respond poorly to in vitro stimulation

Proliferation	Mouse genotype		
	Normal	Scurfy	Transgenic
No stimulation	778	23488	596
Anti-CD3+Anti-CD28	22932	225981	9106

Figure 8

Figure 9



Comparison of FKH^{sf} and JM2 cDNAs. Exon/intron structure is shown (Genomic) as open rectangles (exons) joined by heavy horizontal lines (introns). Coding exons are numbered 1-11 as determined by sequence analysis of FKH^{sf} cDNA; non-coding 5' exons are labelled *a* and *b*. The FKH^{sf}-specific and JM2-specific splicing patterns and resulting cDNAs are indicated above and below the genomic structure, respectively.

<i>N-terminal</i>		<i>ZNF</i>	<i>Mild</i>	<i>Forkhead</i>	<u>Human FKhsf</u>	<u>Mouse FKhsf</u>
					95.8%	82.8%
					83.4%	96.4%

Human and mouse FKH^{sf} proteins are highly conserved.

Figure 10